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Disseminated Bovine Tuberculosis in a Wild Red Fox (*Vulpes vulpes*) in Southern Spain

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ABSTRACT: A wild-caught, adult red fox (*Vulpes vulpes*) from Doñana National Park (southern Spain), in very poor condition, died during anesthesia. At necropsy, the submandibular, retropharyngeal, mediastinal, axillary, mesenteric, and popliteal lymph nodes were enlarged, and the right submandibular and mesenteric lymph nodes had hard, white-colored concretions (calcifications). Multiple white pinpoint foci were observed in the lungs, and abscesses were present in the left dorsal lung lobes. No lesions were seen in tonsils, liver, or spleen. On histopathology multiple tuberculous granulomas, with Ziehl-Neelsen–positive bacilli, were observed in the lung, and multifocal necrotic areas with calcification were present in the submandibular and mesenteric lymph nodes. Nucleic acid amplification from lymph node samples demonstrated the presence of mycobacteria belonging to the *Mycobacterium tuberculosis* complex. One strain was isolated by culture and identified as *Mycobacterium bovis*. The suspected route of infection was oral, probably after repeated scavenging of infected wild ungulate carcasses. This is the first report of generalized bovine tuberculosis (bTB) in a wild canid. This finding raises concerns about bTB as a disease risk for protected species, livestock, and humans in Mediterranean Spain.

Key words: Andalusia, Canidae, carnivore, *Mycobacterium bovis*, scavenger, tuberculosis.

Mycobacterium bovis, the etiological agent of bovine tuberculosis (bTB), has one of the broadest host ranges of all pathogens (de Lisle et al., 2001), including carnivorous mammals. However, canids appear less susceptible to the disease, especially when compared with felids or mustelids. For example, in a 30-yr-long survey in New Zealand, two isolates of *M. bovis* were obtained in dogs in contrast with 76 isolates in cats (de Lisle et al., 1993). Gay et al. (2000) stated that dogs are naturally resistant to *M. bovis*. This may be speculative, but there are few reports of *M. bovis* infection among wild canids (Table 1), even though the family Canidae include scavenging species inhabiting areas where *M. bovis* is endemic (e.g. the red fox [*Vulpes vulpes*] in Europe or North America, or the wild dog [*Lycaon pyctus*] in Africa). Bruning-Fann et al. (2001) found bTB-suggestive gross or histologic lesions in five coyotes (*Canis latrans*) and one red fox; acid-fast bacilli were observed in only two of these coyotes. Delahay et al. (2006) reported gross lesions in one out of 24 bTB culture–positive red foxes. Carbyn (1982) reported enlargement of lymph nodes in two wolves (*Canis lupus*) infected with *M. bovis* and bTB lesions have been reported in captive fennec foxes (*Fennecus zerda*; Himes et al., 1980). In 2005, *M. bovis* was isolated for the first time in culture from a red fox in Spain. This animal came from the Doñana National Park (DNP) and did not have macroscopic or histologic lesions (Martín-Atance et al., 2005).

In the present case, we describe a disseminated disease caused by *M. bovis* in a wild red fox from the Reserva Biológica, in the DNP (southern Spain: 37°0′N, 6°30′W). The fox was an old male captured with a box trap in February 2005, as part of an ecologic research program. The animal was emaciated with poor pelage, skin ulcers, dyspnea, and pale mucosa. The fox was anesthetized with a combination of ketamine (Imalgène®, Merial, France) plus medetomidine (Domtor®, Salud Animal-Pfizer, Spain), and died about 20 min after induction.

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TABLE 1. Summary of reports of *Mycobacterium bovis* infecting wild canids. This summary does not include captive wildlife.

Species	Origin	Prevalence	Lesions[a]	Suspected route of infection[b]	Reference
Coyote	USA	1/23	No	Ingestion of elk (*Cervus elaphus*)	Rhyan et al. 1995
Canis latrans	USA	6/110	M (1), H (3), both (1)	Ingestion of white-tailed deer (*Odocoileus virginianus*)	Bruning-Fann et al. 2001
Wolf	Canada	2/21	M (2)	n.s.	Carbyn 1982
Canis lupus					
Gray fox	USA	1/?	n.s.	n.s.	Patrick 2003
Urocyon cinereo-argenteus					
Red fox	USA	1/6	No	Ingestion of white-tailed deer	Bruning-Fann et al. 2001
Vulpes vulpes	Spain	1/7	No	n.s.	Martín-Atance et al. 2005
	UK	24/756	M (1)	Ingestion of infectious carcasses of cattle or cervids	Delahay et al. 2006
	Spain	2/24	H (1), both (1)	Ingestion of infectious carcasses of wild ungulates	Present study

[a] M=macroscopic, H=histologic, (*n*)=number of cases; unspecific lesions such as "enlargement of lymph nodes" are not included.
[b] n.s.=not specified.

Whole blood was obtained from jugular veins just before death, collected in serum separator tubes, and allowed to clot. The sample was centrifuged and serum was removed and frozen at −20 C until analyzed. The carcass was necropsied and samples from the major viscera were taken. Samples for histology were routinely fixed in buffered formalin and processed in 4-mm paraffin-embedded cassettes; 4-μm cuts were obtained and stained with hematoxylin-eosin and Ziehl-Neelsen (ZN). Polymerase chain reaction (PCR) was used for detection of *Mycobacterium tuberculosis* complex (MTC). Nucleic acid corresponding to MTC in microbiologic culture and in mandibular or retropharyngeal lymph nodes was performed with the use of primers TB1-F (GAA CAA TCC GCA GTT GAC AA) and TB1-R (AGC ACG CTG TCA ATC ATG TA), as described previously (Böddinghauss et al., 1990; Liébana et al., 1996), which amplify a 372–base-pair (bp) fragment of the gene that coded the MPB20 antigen specific for MTC (Cousins et al., 1991). Culture was undertaken with the use of macerates of lymph node tissue suspended in 0.2% buffered bovine albumin, and decontaminated with a benzalkonium substrate (Kubica's method modified by Krasnow) (Casal, 1983). Centrifuged deposits were resuspended in 10% buffered bovine serum and cultured on Middlebrook's 7H11 oleic acid albumin agar medium (Gallagher and Horwill, 1977) and Lowestein-Jensen with pyruvate and without glycerol (Corner and Nicolacopoulos, 1988). The microbiologic identification of mycobacteria was based on tests selected by Wayne and Kubica (1986) in accordance with the methods reported previously (León-Vizcaíno et al., 1990). Antibodies against *M. bovis* were detected in serum with the use of the indirect competition enzyme-linked immunosorbent assay (ELISA) technique. This was developed according to the method described by Harboe et al. (1990) with modifications (Acosta et al., 2000). Antigen consisted of MPB70 protein (Harboe and Nagai, 1984) and an anti–*M. bovis* goat serum was used to measure antigen captured by test and control sera. Positive- and negative-control serum samples were from red fox;

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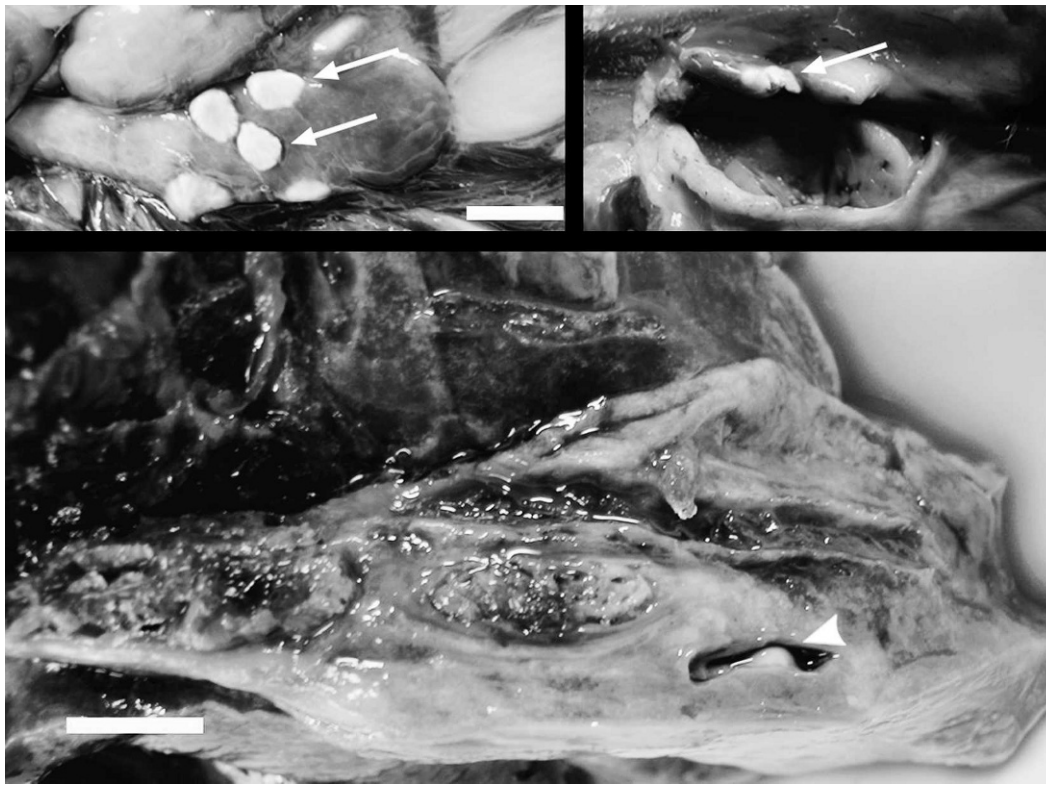


FIGURE 1. Bovine tuberculosis lesions in red fox (*Vulpes vulpes*). Calcification (arrows) of mesenteric lymph node (upper left), and in a section of the submandibular lymph node (upper right); and an abscess (arrowhead) in the left dorsal lobe of the lung (below). Bars=1 cm.

positive serum was obtained from a fox that was experimentally vaccinated with *M. bovis* BCG (see Martín-Atance et al., 2006 for further details).

External examination indicated that this animal was in very poor body condition. Deep ulcers were noted bilaterally in the skin over both ischia. The right ear was torn and the tip was missing. The muzzle was scarred and many teeth were worn down. Submandibular, retropharyngeal, mediastinal, axillary, mesenteric, and popliteal lymph nodes were enlarged. Hard, white concretions (calcifications) were detected in the right submandibular and mesenteric lymph nodes when cut (Fig. 1). No lesions were seen in the tonsils. The heart was enlarged and pale. In the lung, abscesses in the dorsal aspect of the left caudal lobe were present (Fig. 1). Multiple white pinpoint foci were seen in both lungs. Both types of lesions were attributable to *M. bovis* infection. Bronchopulmonary nematodes were observed in the right lung. The stomach was empty of feedstuff. Blood vessels appeared engorged in the intestines. The gall bladder was full and the liver was grossly normal. The spleen was pale. Perirenal adipose tissue was absent and the adrenal glands were enlarged.

In histopathologic examination, the lung contained multifocal nodular accumulations of lymphocytes, macrophages, and some plasma cells, occasionally surrounding necrotic cellular debris or sections of metazoan parasites (granulomas). Acid-fast bacilli were detected in the cytoplasm of some macrophages (two–four bacilli per granuloma). The submandibular lymph node contained scattered giant multinucleated cells and multifocal dark basophil-

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ic concretions (calcifications). The mesenteric lymph nodes contained similar calcifications and multifocal necrotic areas surrounded by lymphocytes, macrophages, and plasma cells. The liver had generalized necrosis. Antibodies against *M. bovis* were detected in sera. Nucleic acid amplification from lymph node samples demonstrated the presence of mycobacteria belonging to the *M. tuberculosis* complex. One strain was isolated by means of culture and identified by biochemical test as *M. bovis*. A concomitant infection with Canine Adenovirus-1 was also detected by means of immunofluorescence [210-34-CAV FITC Conjugate (Anti-Canine Adenovirus polyclonal antiserum conjugated to fluorescein isothiocyanate), VMRD Inc., Pullman, Washington, USA], and competitive ELISA (Canine Hepatitis IgG ELISA, Eurovet Veterinaria Daganzo, Madrid, Spain).

From our review of the literature, this is the first report of disseminated bTB in a wild canid. In the domesticated dog, it appears that natural *M. bovis* infection is one of wide anatomic lesion distribution (Francis, 1958). The generalized distribution of lesions of the disease in the present case may have been due to the advanced age and general debility of the fox, which may have increased the susceptibility of the animal to generalized infection. Tuberculosis may have a clinical course of many months (Kaneene and Thoen, 2004). In agreement with our findings, the six infected coyotes studied by Bruning-Fann et al. (2001) were also adults. Conversely, the cases of bTB in wolves reported by Carbyn (1982) were two littermate pups of about 6 mo of age, both found emaciated because of the *M. bovis* infection. The presence of concurrent infections, such as infectious canine hepatitis (which may have been the cause of the hepatic necrosis), may also have weakened the immune system of the fox and as a consequence predisposed it to generalized bTB.

The distribution of the lesions suggested that the primary route of infection was the digestive tract, probably after repeated scavenging of infected wild ungulate carcasses. This agrees with other reports of bTB in canids that are often associated with high prevalences of bTB among cattle or wild cervid populations (Table 1). Red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and wild boar (*Sus scrofa*) in southern, Mediterranean Spain have been recorded with a high prevalence of bTB (up to 100% in some wild boar populations; Vicente et al., 2006). These species are probably bTB reservoirs in this region of Spain (Vicente et al., 2006). Ungulates from DNP, where the fox was caught, are not an exception (Aranaz et al., 2004). As previously indicated, the infected fox reported by Martín-Atance et al. (2005) also belonged to DNP. In addition, antibodies against *M. bovis* were found in 23% of badgers (*Meles meles*) and 3% of endangered Iberian lynxes (*Lynx pardinus*) in DNP (Martín-Atance et al., 2006). Moreover, in this location three lynxes were found to be *M. bovis* positive, and the isolates showed an identical spoligotyping pattern to the predominant pattern found in domesticated and wild ungulate species in Southern Spain (Aranaz et al., 2004).

Feeding ecology may also explain the differences in the etiologic agent of TB between wild canids and the domestic dog. Although dogs are equally sensitive to *M. bovis* and *M. tuberculosis* experimentally (Francis, 1958), approximately 75% of cases of tuberculosis reported in dogs are caused by *M. tuberculosis* (Forster et al., 1986). Therefore, whereas dogs usually become infected with *M. tuberculosis* by indirect association with humans (Hackendahl et al., 2004), wild canids may become infected by *M. bovis* by the ingestion of infected carcasses.

Carnivores may serve as bTB reservoirs in other countries, for example, badgers in the UK (Delahay et al., 2006), or feral ferrets (*Mustela furo*) in some situations in Australia (Corner, 2006). However, the fox is usually a spillover host, at least in the

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UK (Delahay et al., 2006), and probably in the Iberian Peninsula. So far, another 24 foxes from DNP have been necropsied and only in one other case was a Z-N–positive individual detected (unpubl. data).

The finding of a positive animal of a species with little reported susceptibility to *M. bovis*, such as the red fox, with generalized bTB lesions, suggests that opportunities for infection with *M. bovis* are present in the natural environment in the southern Iberian Peninsula, and that bTB is a threat for the conservation of wild species including the extremely endangered Iberian lynx, that inhabits DNP. In addition, biologists and other professionals working with wild carnivores must take into account the risks of acquiring bTB infection from infected samples and tissues, or from bite wounds.

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